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DEPARTMENT OF TRANSPORTATION INHALATION TEST OF NEUTRALIZED GB HYDROLYSATE IN SPRAGUE-DAWLEY RATS

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14. ABSTRACT The Assembled Chemical Weapons Alternatives (ACWA) Program has been tasked to demonstrate alternative technologies that will safely dispose of assembled chemicals munitions through means other than incineration. The ACWA program is currently investigating GB hydrolysate, a product solution resulting from chemically neutralizing GB with aqueous sodium hydroxide (pH 12.8) as an acceptably treated waste that can be transported offsite for secondary treatment. An acute inhalation toxicity test was conducted on a pH adjusted hydrolysate solution (pH 7.8) to assess the toxicity of the hydrolyzed components, a mix of aqueous soluble organics, metals, and anions, without its corrosive properties. Sprague-Dawley rats were exposed for 1 hr to an aerosol concentration of 3.5 mg/L hydrolysate per Department of Transportation (DOT) guidelines. The rats did not exhibit any signs of either irritation or overt toxicity during either the exposure or post-exposure periods. Further, no mortality occurred within the 14 day post-exposure period, an endpoint of the DOT study. The product solution from the neutralized (pH 7.8) hydrolysate does not appear to pose an acute inhalation hazard.					
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PREFACE

The work described in this report was authorized under Sales Order No. 8VEJW9. The work was started in May 2008 and completed in October 2008. The experimental data are recorded in Laboratory Notebook No. 08-0046.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council."

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QUALITY ASSURANCE

(U) This study, conducted as described in Protocol 08-403, and reported as "DOT Inhalation Test of Neutralized GB Hydrolysate in Sprague-Dawley Rats", was examined for compliance with Good Laboratory Practices as published by the U. S. Environmental Protection Agency in 40 CFR Part 792. The dates of all inspections and the dates the results of those inspections were reported to the Study Director and management were as follows:

<u>Phase Inspected</u>	<u>Date</u>	<u>Reported</u>
Exposure and Study Parameters	13 Aug 08	13 Aug 08
Data and Final Report	26 Feb 09	26 Feb 09

(U) To the best of my knowledge, the methods described were the methods followed during the study. The report was determined to be an accurate reflection of the raw data obtained.



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DEPARTMENT OF TRANSPORTATION INHALATION TEST
OF NEUTRALIZED GB HYDROLYSATE
IN SPRAGUE-DAWLEY RATS

1. INTRODUCTION

Chemical munitions for the nerve agent GB are currently stockpiled at the Kentucky Blue Grass U.S. Army Depot (Richmond, KY). Mandates by Congress stipulate the complete destruction of the chemical weapons (CW) stockpile IAW the Chemical Weapons Convention. The Program Manager for the Assembled Chemical Weapons Alternatives (ACWA) has been tasked by the Department of Defense (DOD) to research and implement the safest means for this destruction, through means other than incineration. The process chosen to destroy GB involves chemical neutralization followed by secondary treatment; either oxidation (on-site) or biotreatment (transportation off-site) (see Figure).¹ Transportation of potentially hazardous material requires a toxicological assessment should an accidental spill and subsequent exposure occur.

This report summarizes the procedures and results of an acute inhalation study on the neutralized GB/NaOH hydrolysate solution (pH 7.8). Although there is some toxicity data on some of the individual components (hydroxides, fluorides, salts), there is little to no inhalation toxicity data on the main reactants (phosphonic acids) or to the reactant mass as a whole. To help fill this data gap, an inhalation exposure was conducted following the guidelines established by the Department of Transportation (DOT) in accordance to the Code of Federal Regulation (CFR) 49, Part 173.132).² Per these guidelines, rats were exposed (whole body) to an aerosol concentration of 2-4 mg/L GB hydrolysate for 1 hr. Information from this testing will be used to assess the inhalation hazard of the material as well as help assign a classification level for transporting the material.

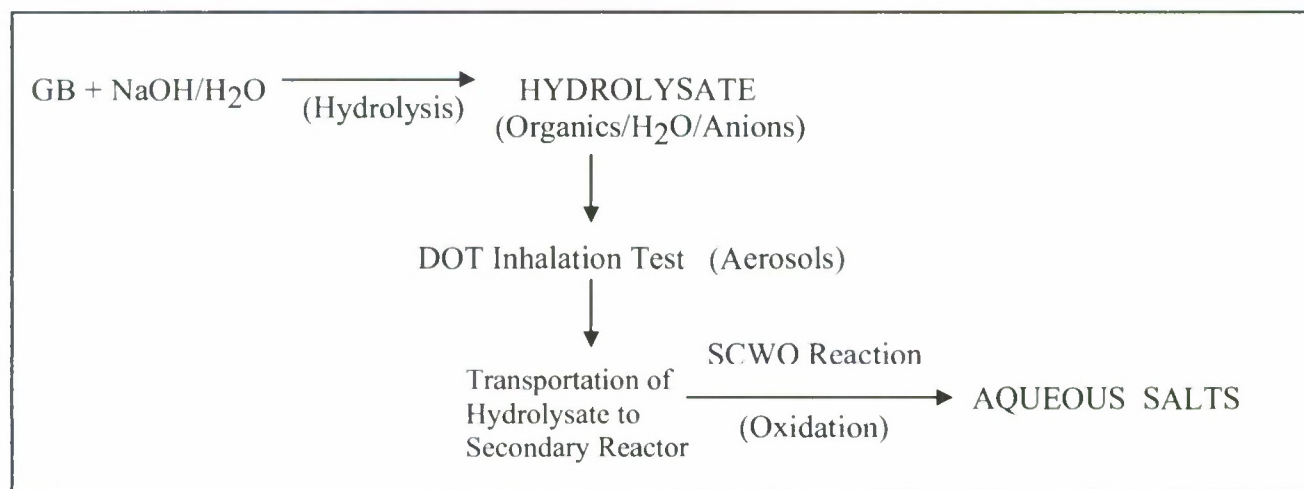


Figure. Proposed GB Neutralization Process and Inhalation Toxicity on Hydrolysate

2. MATERIALS AND METHODS

2.1 Test Material.

The GB hydrolysate test solution was designated as GB/NaOH (GB-8072-1). The test solution was prepared under laboratory conditions at the U.S. Army Edgewood Chemical Biological Center (ECBC) by reacting 7.5 wt% GB (Chemical Agent Standard Analytical Reference Material, CASARM grade, stabilized with tributylamine, CAS# 102-82-9) with 5.7 wt% sodium hydroxide (NaOH) at temperatures ranging from room temperature to 71 °C. The resulting hydrolysate solution was a clear golden brown color with very little precipitate. Due to the caustic characteristics of this material (pH ~12.8), the pH was adjusted down to 7.8 using 1.0 M HCl at 1 day prior to testing. This was to assess the potential toxicity of the reaction products on the animals without excessively harming them due to the hydrolysate's corrosive properties.

2.2 Process Chemistry - Chemical Neutralization Reaction.

The process to chemically neutralize munitions grade GB involves its reaction with aqueous NaOH to produce isopropyl methylphosphonic acid (IMPA) and hydrofluoric acid (HF) (eq 1).³



The HF readily neutralizes in excess NaOH to form sodium fluoride or fluoride ion (eq 2).



The GB degradation product IMPA may undergo a further reaction in water to produce methylphosphonic acid (MPA) and isopropyl alcohol (eq 3). However, this reaction is less likely to produce significant amounts of MPA.



2.3 Animals.

Young adult, male (163-181 g) and female (196-206 g) Sprague-Dawley rats were obtained from Charles River Laboratories, Incorporated (Wilmington, MA). The animals were quarantined and evaluated for general condition and health status. The animals were then identified by permanent marker (tail) and housed in plastic rat cages in the animal holding facility. Housing conditions were maintained at 70 + 5 °F, 30-70% relative humidity (RH) and a 12:12 hr light-dark cycle. Certified rodent diet (Harlan Teklad, Madison, WI) and filtered house water were available *ad libitum*, except during testing.

Prior to testing, all animals were weighed, numbered, and randomly placed into groups. Animal weight ranges on the day of exposure were (214-253 g) for five males and (226-251 g) for five females.

No controls were required for DOT toxicity testing, however, one male rat outside of the exposure group was submitted for serological health monitoring on the day the rats were received.

2.4 Toxicity Testing.

2.4.1 Inhalation Exposure System.

Animal exposures were conducted in a 750 L dynamic airflow inhalation chamber. The chamber flow rate, and test compound feed-rate were determined during the calibration period to achieve an exposure concentration of 2-4 mg/L aerosol. Aerosol samples from the chamber were collected onto filter pads for gravimetric and analytical analysis to characterize and quantitate the chamber concentration. Aerosol particle sizing was determined by drawing chamber air through a cascade impactor followed by gravimetric analysis.

The aerosol generation system, located on top of the 750 L chamber, consisted of a 0.5 L glass reservoir (which contained the test solution), a fluid metering pump (Fluid Metering, Incorporated, Oyster Bay, NY), and a spray atomizer (Spray Atomization Nozzle 1/4 J SS, Spraying Systems Co., Wheaton, IL). Once activated, the fluid metering pump delivered a constant flow rate of the hydrolysate (2.55 mL/min) through a flexible plastic line (~ 8 in. 1/16 in. o.d. x 1/50 in. i.d.) into the spray atomizer. A volume of compressed air (46 psi, 25 L/min) was directed through the atomizer, which sprayed the hydrolysate solution into a respirable-sized aerosol at the chamber inlet. The nominal aerosol concentration in the chamber was calculated by dividing the measured flow rates of the liquid hydrolysate from the measured chamber flow rate. The chamber flow rate was measured with a thermo-anemometer (Model 8570, Alnor, Skokie, IL) before and after exposure.

2.4.2 Acute Inhalation Exposure.

The acute inhalation exposure was set up according to DOT guidelines described in CFR 49, Part 173.132 (10/01/2007 Edition).² These guidelines determine the packing group for poisonous materials (Class 6, Division 6.1) based on toxicity observed from animal exposures to various aerosol concentrations (Table 1). Sprague-Dawley rats (five male and five female) were exposed (whole body) to aerosols from the GB hydrolysate for 1 hr at the highest packing group level (≥ 2 and ≤ 4 mg/L) and observed for 50% lethality within a 14 day post-exposure period. In addition, DOT guidelines stipulate that $> 90\%$ of the particles were within the respirable range ($< 10 \mu$ particle size). No control group animals were required for this testing.

Table 1. DOT Hazard Classification and Packaging Categories for Division 6.1 Mixtures²

DOT Inhalation Toxicity Testing for Aerosols	
<u>Packing Group</u>	<u>Inhalation Toxicity by dusts and mists LC50 (mg/L)</u>
I	≤ 0.2
II	≥ 0.2 and ≤ 2.0
III	≥ 2.0 and ≤ 4.0

2.5 Sample Collection and Analysis.

2.5.1 Aerosol Sample Collection.

Aerosol concentrations of various components of the GB hydrolysate were determined by collecting filter pad samples drawn from the animals breathing zone during exposure. Following sampling, all filter pads were weighed to verify a stable aerosol concentration, then desorbed with an appropriate solvent (water or methanol) for quantitation of various components of the hydrolysate mixture. Multiple types of filters were used to collect these components. Glass fiber filters (Type A/E 1 μm 25 mm, Pall Corporation, Ann Arbor, MI) were used for anion and organics extraction. Teflon[®] [polytetrafluoroethylene (PTFE), 1.0 μm 25 mm, Sterlitech Corporation, Kent, WA] laminated membrane filters were used to collect for metals (Na) and organics. Mixed cellulose ester filters (AAWP 0.8 μm 25 mm, Millipore Corporation, Bedford, MA) were also used to collect for Na analysis. All sampling flow rates were validated by connecting a flow calibrator to the sample line to measure flow rates (DryCal, Model DCL-ML, Bios International Corporation, Butler, NJ).

Filter samples were collected following chamber equilibration (t_{99}), which was attained at 4.5 min into the exposure. A particle size sample was collected at 6-11 min into the exposure followed by filter pad samples collected approximately every 5 min during the 60 min exposure. Filter pad samples were drawn at a rate of 1L/min for 2 min.

2.5.2 Particle Size Sample Collection.

The aerodynamic particle size was measured using a 10-stage cascade impactor (Model 1154, Sierra Instruments, Monterey, CA). Chamber air samples were drawn through the impactor at a rate of 7 L/min. Aerosols drawn through the impactor were collected onto glass fiber substrates beneath each stage. The substrates were subsequently weighed to determine the mass collected at each size range. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (σ_g) were determined by log-normal regression (least squares method) of particle size versus cumulative relative mass.

2.5.3 Fluoride Anion Analysis.

Anion analysis was conducted using an ion chromatograph (Model ICS-2000, Dionex Corporation, Sunnyvale, CA). Separation and quantitation for fluoride was performed on a IonPac AS 18 (4 x 250 mm) analytical and guard column AG 18 (4 x 50 mm) with suppressed conductivity detection. Separation was achieved by using an isocratic potassium hydroxide (30 mM) eluent at a flow of 1 mL/min with an anion self-regeneration suppressor (ASRS Ultra 4-mm). Filter samples were repeatedly rinsed and the fluoride desorbed with 18M Ω deionized water (Millipore Milli-Q purification system, Billerica, MA) into a 25 mL volumetric flask. The hydrolysate solution was diluted 1/2,500 in water for fluoride analysis. A 25 μ L injection was made onto the column for samples and standards. A linear regression fit ($R^2 = 0.9999$) of five fluoride standards (0.8 - 6.0 ppm) injected on the ion chromatograph were used to calculate for the amount of fluoride on each filter sample, as well as in the hydrolysate solution.

2.5.4 Sodium Metal Analysis.

Sodium analysis was conducted using atomic absorption spectroscopy (AAS) (Model 2380, Perkin Elmer, Shelton, CT). Instrumental conditions used an air-acetylene flame with a 3 in. burner head at a wavelength of 589.59 nm. The PTFE (Teflon[®]) and AAWP filters were used for sample collection in the chamber. The PTFE filters had the advantage of an extremely low sodium background, whereas the AAWP filters were hydrophilic filters. Filter samples were desorbed with an acid/water mixture (0.15% nitric acid in deionized water) into a tared trace metal free polypropylene container (DigiTUBE[®], SCP Science, Champlain, NY). The final dilution volume (25-28 mL) was based on a final tare weight after metal desorption. The hydrolysate solution was diluted 1/2,500 in the same acid/water mixture as the filters and standards. Following dilution and filter extraction, a linear regression fit ($R^2 = 0.9993$) of five sodium standards (0.5 - 9.9 ppm) on the AAS were used to calculate the amount of sodium on each filter as well as the amount in the hydrolysate solution.

2.5.5 IMPA Analysis.

The PTFE and glass fiber filters (GF) were used for IMPA sample collection in the chamber. Filter samples were sequentially desorbed and sonicated with HPLC-grade methanol (Fisher Scientific Corporation, Hampton, NH) up to a 25 mL volume with subsequent dilutions (1/625) prior to analysis. The hydrolysate solution was diluted 1/25,000 in methanol for analysis. IMPA was weighed and diluted in methanol to create a stock solution followed by serial dilutions of five IMPA standards (0.10 - 5.0 μ g/mL).

IMPA analysis was conducted on an Agilent 1100 series liquid chromatograph interfaced with an Agilent 6410 triple quadrupole mass spectrometer (MS) (Agilent Technologies, Santa Clara, CA). Injections of 0.5 μ L of extract were made with a constant flow rate of 0.8 mL/min through a Zorbax Eclipse SB-C18 column (2.1 mm x 30 mm, 3.5 μ , Agilent Technologies) housed in a 40 $^{\circ}$ C compartment for chromatographic separation. The solvent gradient program was initially composed of 0% organic phase (0.1% formic acid in methanol) and 100% aqueous phase (0.1% formic acid in deionized water). This was followed by a linear

gradient program increasing to 100% organic phase over a 4 min ramp. The mobile phase was returned to the initial conditions over a 1 min gradient for a total run time of 5 min. Detection was performed using positive ion electrospray ionization with Multiple Reaction Monitoring (MRM) analysis. The conditions for MRM analysis were: fragmentor voltage (80V), capillary voltage (4000V), nitrogen drying gas temperature (350 °C), drying gas flow (11 LPM), and collision energy (4V). Data were collected at unit resolution at a dwell rate of 200 msec for a single precursor to product ion transition of m/z 139 > 97 for IMPA.

The Agilent software package “MassHunter” provided with the MS was used to process and analyze the data. The software allowed automatic analysis of the external standard method based on the analyte area of the peaks at their respective retention times. Automated peak selections were checked to ensure for the proper peak selection, peak shape, baseline evaluation, and presence of interferences. The concentrations of unknown samples were determined using the slope and intercept calculated by linear regression analysis ($R^2 = 0.999$) of the calibration curve.

3. RESULTS

3.1 Neutralized GB-Hydrolysate.

The GB-hydrolysate solution contained a wide variety of organics, metals, and other components (anions/ dissolved solids) as a result of the neutralization process. Analysis on the amount of GB remaining in the hydrolysate was below the sample clearance level (< 20 ppb).⁴ A summary composition of a 7.5% caustic GB hydrolysate, derived from numerous batches for the PM ACWA Demonstration Test Program, shows the approximate amounts of the organic and inorganic constituents, which would typically be present (see the Appendix).

For this study, three major constituents (fluoride, IMPA, and sodium) were targeted for sampling and analysis to characterize the exposure atmosphere. These constituents were the primary reaction products from the hydrolysis reaction (eqs 1 & 2). Analysis of the hydrolysate test solution found that it contained 16,951 $\mu\text{g/mL} \pm 4$ of fluoride, 36,810 $\mu\text{g/mL} \pm 590$ of IMPA, and 22,297 $\mu\text{g/mL} \pm 129$ of sodium. An approximation on the amount of MPA in the hydrolysate was 2,500 $\mu\text{g/mL}$, based on HPLC/MS/MS techniques similar to IMPA analysis; however, unresolved peak separation for MPA did not allow for its quantitation.

3.2 Aerosol Concentration During Exposure.

Particle sizing with the cascade impactor was conducted during the beginning of the exposure (6-11 min). Aerosol filter samples were then drawn at various times throughout the rest of the exposure, targeting various constituents of the hydrolysate (Table 2). All filter samples were also weighed just prior to testing and immediately after they were drawn from the chamber to obtain a relative weight for each sample.

Table 2. Summary of Aerosol Sampling and Component Concentration during 1 Hr Exposure

Sample Time (Min)	Sample/Filter Type	F ⁻ (µg/L)	IMPA (µg/L)	Na (µg/L)	Gravimetric (mg)
6 - 11	Cascade Impactor/GF				
13 - 15	Fluoride/GF	47.9			0.777
18 - 20	IMPA/PFTE		78.3		0.797
23 - 25	Sodium/AAWP			61.4	0.801
28 - 30	Fluoride/GF	47.7			0.745
33 - 35	IMPA/PFTE		81.4		0.790
38 - 40	Sodium/PFTE			57.6	0.771
43 - 45	Fluoride/GF	49.9			0.779
48 - 50	IMPA/GF		83.4		0.789
53 - 55	Sodium/AAWP			59.9	0.835
Mean Fluoride	=	48.5 µg/L ± 1.21;	CV = 2.5 %		
Mean IMPA	=	81.0 µg/L ± 2.58;	CV = 3.2%		
Mean Sodium	=	59.6 µg/L ± 1.94;	CV = 3.3%		
Mean Gravimetric	=	0.79 mg ± 0.025;	CV = 3.1%		

The nominal aerosol concentration in the chamber was 3.5 mg/L. Calculations to determine the nominal concentrations of total aerosol and targeted components in the chamber are shown in Table 3.

Table 3. Nominal Chamber Concentration (Total Aerosol and Individual Components)

$\text{Total Aerosol (mg/L)} = \frac{2.55 \text{ mL/min (feed rate)} \times 1.05 \text{ g/mL (density)} \times 10^3 \text{ (mg/g)}}{770 \text{ L/min (Chamber Flow)}} = 3.5 \text{ mg/L}$	
$\text{Nominal Component } \mu\text{g/L} = \frac{(\text{F}^-, \text{IMPA, Na } \mu\text{g/mL in hydrolysate}) \times (\text{feed rate } 2.55 \text{ mL/min})}{\text{Chamber Flow (770 L/min)}}$	
<p>Where: F⁻ = 16,951 µg/mL; IMPA = 36,810 µg/mL; Na = 22,297 µg/mL</p>	

The mean analytical and nominal chamber concentrations for each of the three components are summarized in Table 4. The percent recovery represents the analytical concentration divided by the calculated nominal concentration.

Table 4. Comparison of Analytical vs. Nominal Chamber Concentration and Percent Recovery

<u>Analytical Concentration</u>	<u>Nominal Concentration</u>	<u>Percent Recovery</u>
F ⁻ = 48.5 µg/L	F ⁻ = 56.1 µg/L	86%
IMPA = 81.0 µg/L	IMPA = 121.9 µg/L	66%
Na = 59.6 µg/L	Na = 73.8 µg/L	81%

3.3 Aerosol Particle Size.

The aerosol MMAD size was 2.78 µg and the σ_g was 3.20 indicating a respirable polydispersed aerosol. More than 90% of the particles were within the respirable range (< 10 µm) for particle deposition in the lung.

3.4 Animal Toxic Signs.

Animals were monitored for toxic signs and behavioral changes during exposure to the GB-hydrolysate aerosol. No toxic signs were noted, including no signs of lacrimation, rhinorrhea, salivation, dyspnea, or any other sign related to organophosphorus exposure. The animals did display an active preening behavior during the first 15 min of the exposure and during the chamber purging. No latent effects were manifest during the 14 day post-exposure period. All animals showed a normal increase in weight and there were 0/10 deaths at the 14 day post-exposure period.

4. DISCUSSION

The reaction of NaOH with GB was the first step of a two-step process to chemically destroy GB. The resulting hydrolysate (step 1) was a caustic liquid containing a complex mix of organics, metals, anions, dissolved solids, and other volatile organic compounds (see the Appendix). An acute inhalation toxicity study was conducted to determine if this process stream would pose an inhalation hazard (DOT Class 6 poison) should either a leak or spill occurred during its transport. An aerosol exposure was favored over a vapor DOT type exposure due to the low vapor pressure of the test material.

In this study, rats were exposed to the highest aerosol inhalation level (Packing Group III, exposure range ≥ 2 to ≤ 4 mg/L) to determine whether an LC50 would occur within a 14 day post-exposure period. The nominal chamber concentration achieved was 3.5 mg/L with a mean recovery of 83% from the measured inorganic constituents. The lower recovery of IMPA

may have been due to partial vaporization of the organic material during the atomization of the solution to produce aerosols. Also, some loss was expected due to adsorption of material onto the chamber walls. Gravimetric samples drawn throughout the chamber exposure demonstrated the stability of the aerosol concentration with a variation of 3.1%.

One of the primary reasons why toxic signs were not displayed by the animals was that the hydrolysate was neutralized from a caustic (pH 12.8) to a more neutral (pH 7.8) solution. Previous toxicity studies have reported that concentrations of NaOH solutions are corrosive to the skin, eyes, and mucous membranes, and inhalation of a mist may cause damage to the upper respiratory tract and to lung tissue, depending upon the severity of the exposure.⁵ Effects of inhalation exposure may vary from mild irritation of the mucous membranes to severe pneumonitis.⁵ Without adjusting the pH, exposure to the hydrolysate containing 5% NaOH would produce effects already reported in the literature as well as possibly mask toxic effects occurring from the phosphonic acids (IMPA, MPA), fluorides, and other constituents that were present. The current DOT classification for a 4-8% NaOH solution is a Category 6, Packing Group II.⁶

The primary organic breakdown product in the hydrolysate was IMPA. There were no reported inhalation toxicity studies for IMPA in the literature. Oral toxicity studies have shown that IMPA possesses low oral toxicity in rats and mice.⁵ Merck reported that it produced only mild skin irritation and no eye irritation in rabbits.^{5,7} A subchronic study on the toxicity of IMPA in drinking water found no statistically significant effects on rats exposed to sodium IMPA, at concentrations ranging from 0-3,000 ppm for 90 days.⁷ The toxicity effects of MPA, the secondary organic breakdown product of GB, are generally more pronounced than IMPA in terms of increased irritancy to the eyes, skin and respiratory tract. However, the aerosol concentration in the chamber, based on the amount of MPA in the hydrolysate (approximately 2,500 µg/mL), would average approximately 8 µg/L MPA, which would most likely not cause any significant irritancy at that concentration level.

The toxicological characterization of the GB hydrolysate (pH 7.8) from the GB/NaOH reaction, as assessed via inhalation exposure, showed no mortality or overt toxicity at the highest DOT exposure level (2-4 mg/L). Also, no mortality occurred during the recovery period, an endpoint for the DOT study. Based on these findings, the neutralized product solution appears to be less toxic than a DOT Class 6 poison (Packing Group III) material as set forth in 49 CFR.

5. CONCLUSIONS

Based on the findings of this study, the following conclusions can be made:

- The aerosol inhalation toxicity of the reaction product from neutralized GB (pH adjusted to 7.8) was less toxic than "Packing Gp III materials" according to biological criteria set forth in Department of Transportation (DOT) CFR 49, Vol 2 (Part 173.132 - 173.133), Class 6, Division 6.1, October 1, 2007).

- The product solution (GB hydrolysate pH 7.8) does not appear to pose an acute inhalation hazard.

- The non-pH adjusted GB hydrolysate (pH 12.8) should follow the current DOT classification for a corrosive sodium hydroxide (4-8%) solution.

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APPENDIX

SUMMARY OF PRIMARY COMPONENTS IN GB HYDROLYSATE*

Agent Related Organics	CAS #	Acronym	Approximate Relative Amount (µg/mL)
Isopropyl methylphosphonic acid	1832-54-8	IMPA	56,000 – 74,000
Methyl Phosphonic Acid	993-13-5	MPA	2,200 - 2,900
Diisopropyl methylphosphonate	1445-75-6	DIMP	2,600-11,000
Tri-n-butyl amine	102-82-9	TBA	1,700-13,000
Isopropyl alcohol	67-63-0	IPA	0 – 2,000
Metals			
Aluminum	7429-90-5	Al	80-90
Arsenic	7440-38-2	As	10
Calcium	7440-70-2	Ca	20-50
Iron	7439-889-6	Fe	40-60
Phosphorus	7723-14-0	P	16,000
Sodium	7440-23-5	Na	26,000 – 28,000
Solids			
Total Suspended Solids		TSS	75 – 1,600
Total Dissolved Solids		TDS	115,000 – 126,000
Other			
Fluoride		F ⁻	8,400 – 9,300
Chemical Oxygen Demand		COD	50,000 – 400,000
Total Inorganic Carbon		TIC	70 – 550
Total Organic Carbon		TOC	21,000 – 50,000
pH			11-13
VOC			
Acetone	67-64-1		0.02 -5.1
Chloroform	67-66-3		0.005-2.6
Methylene chloride	75-09-2		0.01 – 6.6

*Note: Constituents and concentration range are listed from a composite of several hydrolysate samples. Not all components listed and their respective ranges may be present in every hydrolysate sample.